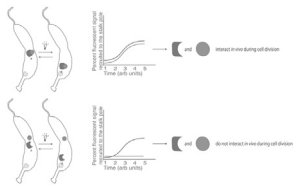
outgrowth in PC12 cells in the absence of growth factors. Intermittent on/off light control reveals a memory effect in ERK-stimulated neurite outgrowth in PC12 cells. The memory effect shows a 45 min off-time threshold, below which a full-speed neurite outgrowth is maintained despite that ERK is gradually turned off. When the off-time is greater than the threshold, the speed of neurite outgrowth decreases with a half time of 2 h as cells slowly lose their memory of prior ERK activation. Interestingly, the 45-min time threshold and the 2-h half time memory are independent of the prior duration of ERK activation. Overall, light-controlled signaling kinetics enables precise dissection of the temporal dimension of signal transduction in cells.

2999-Pos Board B691

Using Optically Reversible Spatial Mutations to Dissect the Asymmetric Developmental Program of a Bacterium

Keren Lasker, Aaron Abraham, W. Seth Childers, Lucy Shapiro.
Developmental Biology, Stanford, Stanford, CA, USA.

Understanding the fundamental basis of cell fate determination is a critical challenge in biology. The Caulobacter bacterium provides the simplest model system for studying asymmetric cell division. Caulobacter's cell cycle regulatory circuit is controlled by a dynamically localized signaling network and targeted proteolysis. This network has been well characterized both genetically and biochemically. However, little is known about the underlying spatiotemporal mechanisms controlling this circuit. Optogenetics techniques offer an unprecedented way to reversibly alter protein localization in vivo in seconds time resolution. We have adapted a light-inducible dimerization system to Caulobacter and introduced light controlled "spatial-mutations" for driving a diffuse protein to specific cellular addresses. We then used these mutations to develop an in vivo spatiotempotal protein interaction assay (opto-conn) to determine whether two proteins are in complex in vivo at a specific cellular address and time point of the cell cycle. Using opto-conn, we are dissecting Caulobacter's regulation pathways by altering the localization and interactions of its key regulatory proteins.



3000-Pos Board B692

Reconstitution of the ENVZ/OMPR Bacterial Signaling System using Supported Lipid Bilayers

Yong Hwee Foo[1], Kabir Hassan Biswas[1], Jay Groves[1,2], Linda J Kenney[1,3].
[1]Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [2]Department of Chemistry, University of California, Berkeley, CA, USA, [3]Department of Microbiology & Immunology, University of Illinois-Chicago, Chicago, IL, USA.

The EnvZ/OmpR two component regulatory system in bacteria regulate the porin genes ompF and ompC in response to the changes in osmolality of the environment. At low osmolality, levels of phosphorylated EnvZ (EnvZ-P) are presumably low, leading to a low amount of OmpR-P. This in turns produces the porin OmpF. On the other hand, at high osmolality, EnvZ-P, and thus OmpR-P levels increase. This represses ompF and produces OmpC. While the general mechanism is known mainly from genetics and biochemical studies, it is difficult to monitor dynamic changes such as the effect of a sudden change of osmolarity and study how that alters the interaction between EnvZ, OmpR and DNA. Thus we employ single-molecule-sensitive fluorescence spectroscopy to study their interaction under differing osmolalities. The cytoplasmic portion of EnvZ (EnvZc) has been shown to be the osmo-sensor (Wang et al., EMBO J, 2012). Thus we purified a cysteine EnvZc mutant (EnvZc294C) and labeled it with Alexa568 at its lone cysteine residue. Using fluorescence correlation spectroscopy (FCS), we showed that EnvZc294C labeled with Alexa 568 (EnvZc294C-A568) binds to OmpR with a dissociation constant Kd of 92 $\pm$ 33 nM in solution. It was also observed that two OmpR molecules bind to a single EnvZc294C-A568 dimer in a cooperative manner. Fluorescence cross-correlation spectroscopy (FCCS) experiments indicated that OmpR labeled with Alexa488 (OmpR-A488) binds to EnvZc294C-A568. With these preliminary results, we are currently anchoring EnvZc294C-A568 onto supported lipid bilayers followed by addition of OmpR-A488. These experiments will explore the effect of changing osmolality, in the presence or absence of ATP and/or DNA, on the interaction between EnvZc, OmpR and DNA by mimicking the organization of proteins in living bacteria. Supported by Mechanobiology RCE from the Ministry of Education, Singapore and VA5IO1BX000372 to LJK.

3001-Pos Board B693

Toward a Spatially-Resolved Model of Metabolism in Dense Bacterial Colonies

John A. Cole[1], Zaida Luthey-Schulten[2].
[1]Physics, UIUC, Urbana, IL, USA, [2]Chemistry, UIUC, Urbana, IL, USA.

An individual cell's metabolic behavior is dependent on several factors, including both its protein-expression state as well as the availability of nutrients in its local environment. These two factors are not always independent; several transcription and translational regulatory systems allow the cell to respond to changes in its environment by adjusting the expression of metabolic enzymes, and thereby shifting its usage among different substrates. By integrating two methods for modeling whole cell phenomena, namely time-dependent reaction-diffusion master equation (RDME) sampling and steady-state flux-balance analysis (FBA), we are able to study how the competition for resources among bacteria in a dense colony gives rise to local micro-environments, and how these micro-environments in turn give rise to drastically different metabolic behaviors among cells. We find, in particular, that cooperative behavior can emerge among cells within the colony. Cells in the anaerobic interior of the colony partially metabolize glucose to acetate, which can then be taken up and metabolized by cells on the more aerobic periphery of the colony.

3002-Pos Board B694

Single-Molecule Super-Resolution Imaging of TcpP Dynamics in Vibrio Cholerae in Response to Virulence Pathway Deactivation by Increased Cell Density

David J. Rowland[1], Hannah Tuson[2], Victor DiRita[3], Julie S. Biteen[2].
[1]Biophysics, University of Michigan, Ann Arbor, Ann Arbor, MI, USA, [2]Chemistry, University of Michigan, Ann Arbor, Ann Arbor, MI, USA, [3]Microbiology and Immunology, University of Michigan, Ann Arbor, Ann Arbor, MI, USA.

The agent of the epidemic human disease cholera is Vibrio cholerae, a bacterium which produces cholera toxin via a virulence pathway that has previously been described biochemically. Various environmental stimuli affect this pathway, which is halted when the cell density is low. One virulence pathway component, TcpP, is a transcription activator that acts in concert with other membrane proteins, ToxS, TcpH and ToxR to regulate ToxT expression. TcpP regulation is itself mediated by various cellular signaling pathways. One popular model for the life of each TcpP molecule suggests that TcpP is coupled to a complex made up of ToxR, TcpH and DNA during transcription, and that TcpP is decoupled from this group upon the deactivation of the virulence pathway before degradation by regulated intramembrane proteolysis. This volume change of the diffusing TcpP particle should cause a measurable change in the rate of diffusion as predicted by the Einstein-Stokes equation. Here, we use single-molecule super-resolution fluorescence microscopy to measure the motion of individual TcpP molecules labeled with the photoactivatable fluorescent protein PAmCherry in live V. cholerae cells. Furthermore, we prepare our samples for imaging within an agarose microfluidic device, in which we set up a linear gradient of spent media inside to simulate increasing cell density. By imaging single cells at different signal concentrations on a single slide, variations in preparation or initial cell state will be decoupled from the effect of cell density on the mobility of TcpP, and we observe changes in TcpP-PAmCherry diffusion that we explain in the context of differences in the virulence pathway activity level.

3003-Pos Board B695

Growing Yeast into Cylindrical Colonies

Clément Vulin, Pascal Hersen.
Université Paris Diderot, Paris, France.

Micro-organisms often form complex multicellular assemblies such as biofilms and colonies. Understanding the interplay between assembly expansion, metabolic yield, and nutrient diffusion within a freely-growing colony remains challenging. Most data available about micro-organisms are from planktonic cultures, due to the lack of experimental tools to observe multicellular assemblies. Here, we propose a method to tackle this problem by constraining the growth of yeast colonies into simple geometric shapes such as cylinders. To this end, we designed a simple, inexpensive, versatile culture system to control the location of nutrient delivery below a growing colony. Under such culture conditions, yeast colonies grow vertically and only at the locations where nutrients are delivered. Colonies increase in height at a steady growth rate which is inversely proportional to the cylinder radius. We show that the vertical growth rate of cylindrical colonies is not defined by the single cell division rate, but rather by the colony metabolic yield. This contrasts with cells in liquid culture, in which the single cell division rate is the only parameter that defines the population growth rate. This method also provides a direct, simple method to estimate the metabolic yield of a colony. Our study further demonstrates the